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Genetic Variants of k-Casein and β-Lactoglobulin Genes and Their Association with Protein and Milk Components of Holstein Friesian Cows at Small Farmers in Lembang, West Java

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Abstract

Genetic variants of CSN3 and LGB genes and their effects on protein and milk components were studied in Holstein Friesian at small dairy farmers in Lembang District, West Java, Indonesia. Allelic variants were identified by PCR-RFLP technique using restriction enzymes of *Pst I* for the CSN3 gene and *Hae III* for the LGB gene. The CSN3 gene was dominated by AB genotype. Milk protein was not affected by genotypes of the two genes. Only fat content was significantly affected ($P < 0.05$) by the CSN3 gene with AB cows having the highest fat to AA and BB cows (3.76% vs. 3.26% and 3.34%).

Keywords: CSN3 gene; LGB gene; milk protein; and milk component.

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1. Introduction

Milk with high protein yield is required as sources of good nutrition especially for children under five and school ages to provide better growth and intelligence. Milk protein with a good composition is useful to overcome health problems, such as hypoalergen on milk. High protein content in milk is also required during milk processing to produce good quality of dairy products. Some protein genes are identified important of having direct effects for high milk protein content especially in dairy cattle. Molecular based selection through the identification of genetic polymorphism of major protein genes can be used to gain genetic improvement of milk protein yield. Better milk quality through the increasing protein and other components will give higher selling prices of fresh milk for small farmers, so it can ensure the sustainability of their dairy cattle business.

Milk protein content in dairy cattle is basically controlled by two groups of major protein genes, namely casein and whey. Casein is the largest milk protein components (78 - 82%) controlled by the only four casein genes. In regarding to physical structure, the four genes have a successive sequence of αs1-casein (αs1-CN), β-casein (β-CN), αs2-casein (αs2-CN) and κ-casein (κ-CN). These respective genes are also known as CSN1S1, CSN2, CSN1S2 and CSN3 with their location very close together, along 250kb at chromosome e6 in cattle (BTA 6q31) [1]. Due to the closing location of these four casein genes lead them very often be inherited as a cluster, seemingly like as a single locus. Casein genes therefore are potential to be explored for molecular selection for genetic improvement on milk protein yield and milk protein composition in dairy cattle [2, 3].

Whey as another group of milk protein components is in the forms of granule or globular. Whey protein content in dairy cattle is about 20%. Its components consist of β-lactoglobulin, α-lactalbumin, Bovine Serum Albumin (BSA), Immunoglobulin and Lactoferrin [4]. In ruminant milk, β-lactoglobulin is one of major proteins and this protein in dairy cows is about 7-12% of the total milk protein [5]. β-lactoglobulin is a group of lipocalinproteins that can bind hydrophobic molecules and plays an important role in fat metabolism [4].

This study was aimed to study genetic variants of -casein gene and β-lactoglobulin gene and their influences on milk protein and other milk components of HF lactation cows from small farmers at North Bandung Milk Collecting Unit (BMCU), Lembang District, West Java. A comparison was conducted to those of HF lactation cows kept under an intensive management at Cikole Dairy Cattle Breeding Station (DCBS) located in Lembang, West Java.

2. Materials and Method

2.1. Location and Sample

Holstein Friesian (HF) lactation cows as research samples were raised by a number of small dairy farmers from two villages, namely Pasar Kemis (Location1) and Cilumber (Location2), under the guidance of North Bandung Milk Collecting Unit (BMCU) in Lembang Distict, West Java. Animals were commonly raised at a small scale under semi-intensive management. A comparison study was done for HF lactation cows raised at Cikole Dairy Cattle Breeding Station (DCBS). Animals in this location were reared in a large scale under an intensive management. This research was conducted from 2008 to 2011.

Blood samples were collected for HF lactation cows from Location1 (60 hd) and Location2 (58 hd) as well as for HF cows from DCBS (55 hd). Blood samples were

collected via jugular vein. Milk samples were collected based on one day milk test, as a result of the amount of morning and noon milk production. The observed HF lactation cows were at physiological status between 1-6 lactation months and 1-5 lactation periods. Genotyping blood samples briefly consisted of DNAextraction, amplification by PCR of DNA fragments of κ-casein and β-lactoglobulin genes. Identification of allelic variants used RFLP method by cutting the PCR product of DNA fragments of each protein gene.

Primers used for DNA amplification of the κ-casein gene were for [6]:

Forward 5'AAA TCCTCACTACCAATACC-3'

Reverse 5'CTTCTTTGACTCTGTCTTAG-3'

whilefor the β-lactoglobulin gene used primers for [7]:

Forward 5'GCTCCCGGTATATGACCACCCTCT3

Reverse 5'TGTGCTGGACACCGACTACA AAAA3

The k-casein gene was cut by Pst1enzyme, while the β-lactoglobulin gene was cut by Hae III enzyme.

Milk protein content was analyzed by formoltitration, at which p as the amount of NaOH used for titration of milk sample and q as the amount of NaOH used for blank titration. Analysis was also done for fat, solid non fat (SNF) and dried matter (DM) components. Milk Protein and other milk components in these analyses were conducted according to the guidance of Codex Alimentarius [8].

2.2. Data Analysis

Frequencies of genotype and allele were calculated referring to formula of Nei and Kumar [9]. Genetic diversity was estimated based on values of heterozygosity observation according to formula of Weir [10]. Study of association of variant genotypes of each protein gene on individual milk components was done by general linear model using SAS 9.1 software). Fixed variables were considered for lactation length (months), lactation period and individual protein genes of the κ-casein gene and the β-lactoglobulin gene.

3. Result and Discussion

3.1. Genotyping and Frequency of Protein Genes

Identification of variant genotypes of the κ-casein gene using PCR-RFLP method in the observed HF lactation cows resulted in three genotypes, namely AA, AB and

Table 1: Frequency of genotype, allele and value of heterozygosity of k-Casein gene and β-Lactoglobulin in HF cows by location.

Loc.	Gene	Genotype			Allele		Heterozygosity	
		AA	AB	BB	A	B	He (Ob)	He (Ex)
Loc. 1	CSN3	0.317	0.650	0.033	0.531	0.469	0.661	0.478
	LGB	0.083	0.583	0.333	0.529	0.471	0.703	0.483
Loc. 2	CSN3	0.241	0.672	0.086	0.870	0.130	0.852	0.502
	LGB	0.017	0.828	0.155	0.735	0.265	0.630	0.472
Both	CSN3	0.280	0.661	0.059	0.911	0.089	0.634	0.461
	LGB	0.051	0.703	0.246	0.621	0.379	0.786	0.488
DCBS	CSN3	0.127	0.818	0.055	0.908	0.092	0.619	0.451
	LGB	0.055	0.655	0.291	0.568	0.432	0.654	0.469
All	CSN3	0.231	0.711	0.058	0.910	0.090	0.676	0.457
	LGB	0.052	0.688	0.260	0.604	0.397	0.736	0.468

BB.AA genotype and BB genotype were characterized by the presence of the only one fragment, corresponding to the fragment lengths of 152bp and183bp respectively. AB genotype had two restriction base sites with the resulting of two fragments of 152bp and183bp. Result from this study was consistent with a previous study on genetic variants of the κ-casein in dairy cows [6]. The k-casein gene of HF cows from this study therefore resulted in two alleles, namely A and B. The occurrence of Aallele and B allele was due to the existing two point mutations at the fourth exon [11, 12].

Both A and B alleles of the κ-casein gene have different amino acids at 136 and 148 base positions. At the position 136 was the amino acid of Thr (ACC) changed by Ile (ATC) while at the position 148 was the amino acid of Asp (GTA) replaced by Ala (GCT) [13]. Three genotypes (AA, AB, and BB) resulted from HF cows in this study were accordance to the previous study by reporting the two A and B alleles of the k-casein gene as common alleles in *Bos taurus* dairy breeds [14]. Table 1 shows that frequencies of the AB genotype of the k-casein gene of HF cows from Location1(0.65) and Location2 (0.67) were very high, resulting frequency of the AB genotype was also high (0.66) in both locations. Moreover, frequencies of the AA and BB genotypes were low, with frequency of the BB genotype was the lowest. The same situation was found at DCBS, with frequency of the BB genotype was also very low. The results proved as a nearly indication of a limited number of the existing HF females having the BB genotype in dairy cattle center in Lembang District of West Java. Higher frequencies of the AB and AA genotypes resulted in higher frequencies of the A allele than those of the B allele as found in both location (0.91 vs.0.09) and all location (0.91 vs.0.09). The same evidence occurred in DCBS with the A allele with a higher frequency over the B allele.

Amplification of the β-lactoglobulin gene produced a fragment length of 247 bp. Karimi *et al.* [7] reported that AA genotype was identified with two fragments, i.e. 148

Table 2: Average of milk component (%) and milk production (lt) of HF cows by location.

Location	Sample	Milk	Fat	SNF	Protein	D. Matter
Loc. 1	60	14.89 ± 4.58^{b}	3.40 ± 0.70	8.21 ± 0.38	2.77 ± 0.29	11.64 ± 0.89
Loc. 2	58	11.96 ± 3.65^{a}	3.54 ± 0.84	8.21 ± 0.53	2.85 ± 0.38	11.80 ± 1.02
Both	118	13.45 ± 4.38^{b}	3.47 ± 0.77	8.21 ± 0.46	2.81 ± 0.34	11.72 ± 0.96
DCBS	55	10.47 ± 3.22^{a}	3.22 ± 0.46	8.28 ± 0.25	3.12 ± 0.12	11.50 ± 0.49
All	173	12.50 ± 4.27	3.39 ± 0.70	8.23 ± 0.40	2.91 ± 0.32	11.65 ± 0.84

Description: Sample in head; same letters in a similar column showed no difference (P > 0.05).

bp and 99 bp; BB genotype with three fragments, i.e. 74 bp, 74bp and 99bp; whereas AB genotype with 3 fragments, i.e. 148 bp, 99 bp, and74 bp. Enzyme Hae III cut at two base sites at positions of 74 and148 bp of PCR products resulting three fragments of 74 bp, 74 bp and 99 bp, expressed as BB genotype. Mutation type identified was substitution mutation for the base changes from purine (AG) orpyrimidine (TC) into cytosine or thymine. This point mutation resulted in the changing expression of amino acid during a translation proces of which valine being translated into alanine.

Similar to the k-casein gene, the β-lactoglobulin gene across location also generated very high frequencies for AB genotype, lower frequencies for BB genotype, but the lowest ones for AA genotype. Higher frequencies of the AB and the BB genotypes resulted in high frequencies of the A allele against those of the B allele in the observed HF cows. HF cows raised in small farmers for both location had the A allele frequency was much higher against the B allele one, namely 0.91 vs. 0.09, while those in all location were 0.60 vs. 0.40. High frequency of the A allele in the β-lactoglobulin gene was also found in HF cattle from DCBS.

Bob *et al.* [15] also gained three genotypes (AA, AB, and BB) in the β-lactoglobulin gene in HF cattle from some areas in United States, thus produced two alleles (A and B). The AB genotype frequency (0.476) was higher against the BB (0.399) and AA (0.125) ones.

3.2. Milk Components

Results of analysis of individual milk components providing protein, fat, solid non fat (SNF), and dried matter (DM) of the observed HF cows were quite good (Table 2). The contents of all these four milk components exceeded minimum limits as specified by ISO3141.1:2011 for fresh milk of dairy cattle in Indonesia. Minimum values specified were 3.0% for fat, 2.8% for protein and 7.8% for SNF. The exception was for protein content of HF cows in Location 1 that was a slightly lower than the SNI provision.

Daily milk production of HF cows in Location1 was higher (P < 0.05) to that in the Location2, but other individual milk components between the two were almost the

same (P > 0.05). Daily milk yield of HF cows in both location (small farmers) was higher (P < 0.05) than that of HF cows in DCBS, respectively13.5 lt and10.5 lt. This might be due to HF cows in the latter were mostly between mid to late lactation months.

3.3. Association of Variant Genotypes of Protein Genes on Milk Components

Effects of variant genotypes of the k-casein gene and the β-lactoglobulin gene on individual milk components of HF cows in this study are presented in Table 3. In general, the k-casein and the β-lactoglobulin genes presented unclear effects on most milk components. However, there was a tendency that the AA genotype of both protein genes gave better milk and other dairy components than the two others. Nevertheless, almost all of variant genotypes of the two protein genes did not significantly affect protein content and other individual dairy components.

An exception was found for significant effect (P < 0.05) of the k-casein variant genotypes on milk fat content under small farmers in both location. The AA cows produced fat content (3.76%) was higher against the AB (3.26%) and the BB (3.34%) cows. Daily milk production in both location was also significantly (P < 0.05) influenced by the β-lactoglobulin variant genotypes. The AA cows resulted in a higher milk production (17.6 lt) against the AB (12.8 lt) and the BB (13.7 lt) ones.

Results of this study were consistent with thestudies in *Bos taurus* dairy cattle conducted by some other researchers. Lin *et al.* [16], which examined the influence of κ-casein genotypes on milk protein per lactation, noted there was no significant effect (P > 0.05) among the three genotypes of AA, AB and BB. However, Eennaam and Medrano [17] reported the BB cows produced the highest protein yield, whereas the AB cows produced milk protein nearly twice against the AA cows. The BB cows produces the highest protein (149 kg), whereas AA and AB cows produces proteins almost equal (142 kg and 143 kg). Significant relationships of the β-lactoglobulin genotypes to either milk composition or milk production were reported by several studies. The B allele had an effect on increasing whey protein content in milk of dairy cattle [15]. Ojala *et al.* [18] reported that the β-lactoglobulin variant genotypes resulted in variant phenotypes on both milk protein content and production, as well as on milk fat percentage in dairy cattle.

4. Conclusion

k-casein gene and β-lactoglobulin gene had the highest frequency of the AB genotype than the two homozigotes of AA and BB genotypes. A fairly good indication of control of the k-casein gene on milk fat content and the β-lactoglobulin on milk production in

Table 3: Effect of variant genotypes of k-Casein and β-lactoglobulin genes on milk component (%) and milk yield (lt) in HF cows by location.

Loc.	Gene	Genot.	N	Milk	Fat	SNF	Protein	D. Matter
Loc.1	CSN3	AA	19	16.86	3.49	8.25	2.73	11.68
		AB	39	16.01	3.35	8.09	2.72	11.50
		BB	2	15.81	3.23	8.17	2.77	11.59
	LGB	AA	5	18.05	3.47	8.07	2.62	11.52
		AB	35	14.73	3.42	8.23	2.83	11.60
		BB	20	15.89	3.35	8.21	2.78	11.65
Loc. 2	CSN3	AA	14	12.89	3.63	8.33	2.82	12.05
		AB	39	12.83	3.48	8.25	2.85	12.36
		BB	5	11.77	3.34	8.45	3.01	12.50
	LGB	AA	1	15.26	3.78	8.49	2.96	13.42
		AB	48	11.20	3.58	8.24	2.93	11.92
		BB	9	11.03	3.30	8.29	2.79	11.56
Both	CSN3	AA	33	15.49	3.26^{a}	8.22	2.74	11.56
		AB	78	14.77	3.76^{b}	8.17	2.78	11.77
		BB	7	13.84	3.34^{a}	8.22	2.85	11.70
	LGB	AA	6	17.63^{b}	3.52	8.22	2.76	11.78
		AB	83	12.77^{a}	3.52	8.22	2.86	11.77
		BB	29	13.71^{a}	3.33	8.17	2.76	11.47
DCBS	CSN3	AA	7	13.24	3.21	8.01	2.95	11.37
		AB	45	13.49	3.23	8.04	3.00	11.37
		BB	3	11.89	3.11	8.15	3.07	11.4
	LGB	AA	3	14.44	3.69	7.91	2.95	11.46
		AB	36	12.22	3.22	8.12	3.02	11.28
		BB	16	11.96	3.13	8.17	3.05	11.38
All	CSN3	AA	40	14.61	3.26	8.21	2.80	11.56
		AB	123	13.70	3.44	8.18	2.89	11.72
		BB	10	12.49	3.35	8.27	2.92	11.77
	LGB	AA	9	15.80	3.58	8.20	2.85	11.82
		AB	119	12.25	3.41	8.24	2.89	11.70
		BB	45	12.76	3.30	8.22	2.86	11.54

Description: Sample in head; different letters in a similar column showed significant difference ($P < 0.05$).

small farmers might open the opportunity to be considered in a GAS program on milk fat content and milk production in HF dairy cows.

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